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PRINCIPAL INVESTIGATOR: Erika J. Mitchell, M.D.

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Nashville, TN 37232

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Erika J. Mitchell, Patrick Norris, Judith Jenkins, John Morris

E-Mail: erika.j.mitchell@vanderbilt.edu; erimitchell@lumc.edu

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14. ABSTRACT

Purpose, scope, significant findings, progress Heterotopic ossification has been associated with numerous factors, however, there has been no conclusive evidence that a given environmental element is causative. We propose that certain individuals are genetically predisposed toward an altered sympathetic response to trauma which not only contributes to post-traumatic morbidity but also the formation of heterotopic bone. We have genotyped 2869 patients admitted to a Level I trauma center ICU for alleles that may be associated with bone healing, autonomic regulation and inflammation. Our preliminary results in a subset of 1313 patients have shown an association of a minor allele of the β2 adrenergic receptor with HO formation. Furthermore, the patients who formed HO also were more likely to have had a prolonged ICU stay and days on a ventilator independent of a higher ISS score which was also associated with HO. Head injury, as defined by the AIS head score, however, was inversely correlated with HO. We have now completed the data collection on 244 SNPs in 2869 individuals and are awaiting final analysis of this data.

15. SUBJECT TERMS
Heterotopic Ossification, Single Nucleotide Polymorphysms, Genetics, Fracture Healing

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INTRODUCTION

Heterotopic ossification (HO) is a complication of spinal cord injury and traumatic limb injuries in both civilian and military populations. The incidence is greater in the military population for unknown reasons. Factors that have been studied as potential causes of HO include head injury, ventilator days, traumatized soft tissue and injury severity have not been conclusive.[1-4] Our data has demonstrated that head injury, believed to be a major risk factor for HO formation, is inversely correlated with HO in our civilian trauma population. We suggest that the cause is not entirely environmental, rather, it is a genetic predisposition that in addition to injury leads to an excessive response to musculoskeletal injury resulting in HO. The greater systemic response to injury in a subset of patients that leads to conditions such as acute respiratory distress syndrome and increased systemic inflammatory response scores may be related to the excessive healing response to musculoskeletal injury that leads to HO.[5-8] We have obtained serum samples from 2869 patients admitted to a Level I trauma center for genetic analysis. The samples have been genotyped for 244 SNP polymorphisms that may be associated with variations in the systemic human response to trauma. Of these patients, 431 had femur fractures with a minimum of 6 weeks of radiographic followup. These radiographs were examined for an excessive heterotopic fracture healing response.

BODY

Previously we had reported on 1313 patients, 125 had evidence of HO (11%). HO was inversely correlated with traumatic brain injury. Injury severity score, ventilator days, ICU days and total hospital days positively correlated with HO formation independent of one another (Aim 2.1, Table 1). In this same group of patients, the CC allele of a polymorphism coding for a β2 adrenergic receptor was associated with HO formation (Aim 1.4, Table 2). This supports the possibility that a differential systemic response to trauma in some individuals is also an underlying cause of heterotopic bone formation.

Genotyping has now been completed on 2869 patients for 244 SNPs. Four hundred and thirty one of these patients had femur fractures and were admitted to the Trauma ICU at Vanderbilt University Medical Center. Out of the 431 patients with femur fractures, 247 met the inclusion criteria of minimum 6 week radiographic followup, Evidence of HO on plain radiograph was seen in 57 (23%) of these patients (FIGURE 1). In addition, data has been collected from the Trauma Registry of the American College of Surgeons (TRACS) to include environmental factors. Our original proposal was to genotype for 36 SNPs in a population of 1200 patients, 200 HO and 1000 no-HO (Aim 1.2). We have been able to increase the number of polymorphisms genotyped due to changes in the technology in our core labs. However, due to the changes in our research team, we chose to limit our radiographic search to femur fractures only. While this decreases the number of patients in the sample size, it also decreases variability from fracture types and treatments which may limit confounding factors due to anatomy, differential surgical techniques and degrees of soft tissue injury. Data collection is now complete but we are still awaiting the analysis.

The changes in our research team have led to the loss of the member who could perform the multivariate analysis. Nonetheless, we are continuing to pursue this albeit much delayed and outside of the research award period. We are attempting to identify an individual and funding to take this to completion for the data analysis as we feel that there is merit to our hypothesis and the data has been collected. We do expect to retain this individual and we will send a supplemental report with our final results including any publications that result from the data as soon as this is achieved.

KEY RESEARCH ACCOMPLISHMENTS

1. Data has been compiled on 2869 individuals including:
    1. AIS Head
    2. ISS
    3. Ventilator days
    4. ICU days
    5. Hospital days
    6. Gender
    7. Age
    8. Radiographic results

2. Genotyping is complete on this population. The 244 SNPs code for the following 32 genes:
    1. Leptin
    2. Leptin receptor
    3. C-reactive protein
    4. Complement Factor H
    5. WNT 3A
    6. WNT 10B
    7. IL1A
    8. IL1B
    9. IL6
    10. ACVR1
    11. GSK3α
    12. GSK3β
    13. Bone sialoprotein
    14. Secreted phosphoprotein 1
    15. β2 Adrenergic receptor
    16. β3 Adrenergic receptor
    17. α1 Adrenergic receptor 1A
    18. α1 Adrenergic receptor 2A
    19. α1 Adrenergic receptor 1B
    20. Tumor necrosis factor
    21. Vascular endothelial growth factor A
    22. Toll like receptor 4
    23. Exostosin 2
    24. LRP5
    25. LRP6
    26. Vitamin D receptor
    27. BMP2
    28. BMP4
    29. Sclerostin
    30. Frizzled 9
    31. FOXO1
    32. FOXO3

3. Radiographs for these individuals have been examined for HO around fractures of the femur
    1. 57 determined to have HO out of 247 (23%) consistent with civilian data

REPORTABLE OUTCOMES

Publications

Mitchell, E.J., Canter, J., Norris, P., Jenkins, J., Morris, J.A. *The Genetics of Heterotopic Ossification: Insight into the Bone Remodeling Pathway.* J Orthop Trauma, 24(9):530-533, 2010.

Presentations

Mitchell, E.J., Morris, J.A., Norris, P., Canter, J., Jenkins, J., *Injury Severity But Not Head Injury Associated with Heterotopic Ossification in Patients Admitted to a Level 1 Trauma Center.* (Poster) Orthopaedic Trauma Assoc., Annual Meeting 2011.

Mitchell, E.J., Morris, J.A., Norris, P., Canter, J., Jenkins, J., *Genetic Predictors of Heterotopic Ossification: Insight into the Bone Remodeling Pathway.* (Invited Presentation) War Extremity Injuries Symposium, AAOS, January 2010

Mitchell, E.J., Morris, J.A., Norris, P., Canter, J., Jenkins, J., *Genetic Predictors of Heterotopic Ossification: Insight into the Bone Remodeling Pathway.* (Podium Presentation) Orthopaedic Trauma Assoc., Annual Meeting 2009.

CONCLUSION

We have completed data collection including genotyping, radiographic examination, and environmental factors on 2869 patients admitted to the Trauma ICU at Vanderbilt University Medical Center. Our preliminary results from a subset of this data have shown a relationship between a polymorphism of the β2 Adrenergic Receptor and the development of HO. Our environmental data has shown that a greater degree of total injury severity but *not* head injury is associated with HO formation. We use the Abbreviated Injury Score (AIS) for head injury instead of the Glascow Coma Scale (GCS) which is a more specific indicator of traumatic brain injury. The GCS on admission has inaccuracies due to intubation and medication of a patient in the field which obscures the ability to assess mental status.

The dataset that has now been completed is more thorough and expected to reveal more relationships. If the β2 Adrenergic Receptor again shows a correlation, this would support our hypothesis that an altered systemic sympathetic response to trauma in certain individuals is a potential factor in the formation of HO. In our preliminary data, patients with longer ICU stays and ventilator days had an increased risk of HO independent of ISS. This also supports our hypothesis. Despite similar injury profiles and demographics, some patients recover without significant complication while others suffer from acute respiratory distress syndrome, systemic inflammatory response syndrome and/or sepsis. We assert that this is a phenomenon dictated by a genetically determined response to trauma that differs across individuals. A better understanding of the underlying causes of this response not only will allow us to better prevent HO but also potentially prevent other morbid events after severe traumatic injuries.

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SUPPORTING DATA

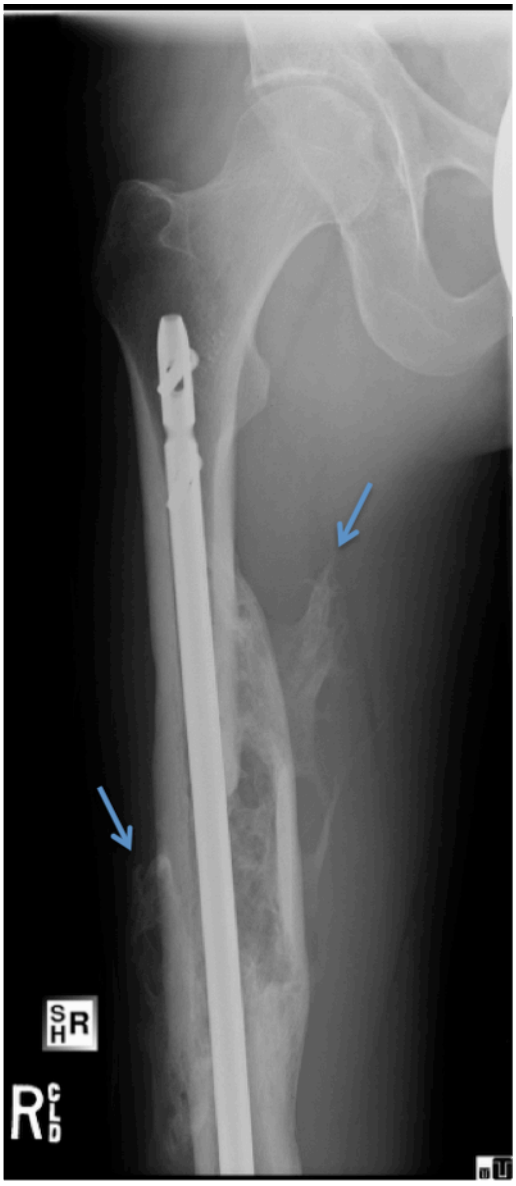

FIGURE 1: Femur fracture with heterotopic bone formation (arrows) as demonstrated by 'flares' of bone away from the site of injury and heterogeneous density compared to typical callus formation at 12 weeks post-injury.

	No HO	HO	p-Value
Age	40.67 ± 17.89	42.69 ± 16.38	0.087
ISS	24.84 ± 12.08	28.35 ± 11.75	**0.003**
AIS Head	2.13 ± 1.84	1.70 ± 1.80	**0.016**
Hosp Days	12.81 ± 12.84	18.42 ± 17.28	**<0.001**
ICU Days	5.61 ± 7.32	8.14 ± 8.75	**<0.001**
Vent Days	4.66 ± 7.11	6.60 ± 6.91	**<0.001**

TABLE 1: Age did not show any relationship with HO. AIS Head score was inversely correlated with HO formation. ISS, Hospital Days, ICU Days, and days on a ventilator were associated with the development of HO.

Polymorphysm	p - value
ADBR2 GG	0.421
ADBR2 GC	0.153
ADBR2 CC	**0.039**
TLR4 CC	0.210
TLR4 CT	0.142
TLR4 TT	0.220
CFH CC	0.161
CFH CT	0.826
CFH TT	0.209

TABLE 2: One of the polymorphisms for the β2 adrenergic receptor was positively associated with the development of HO.

LIST OF PERSONNEL

Erika J Mitchell, MD (PI)
Loyola University Medical Center, Maywood, IL
Dept of Orthopedic Surgery and Rehabilitation

John A Morris, MD (co-PI)
Vanderbilt University Medical Center, Nashville, TN
Department of General Surgery

Patrick Norris, PhD
Vanderbilt University Medical Center, Nashville, TN
Department of General Surgery

Jeffery Canter, PhD (2008-2010)
Vanderbilt University Medical Center, Nashville, TN
Department of General Surgery

Judy Jenkins, RN
Vanderbilt University Medical Center, Nashville, TN
Department of General Surgery

David Taber, MD (2010)
Vanderbilt University Medical Center, Nashville, TN
Department of Radiology